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REVIEW

# Experimental vaccines in animal models for schistosomiasis

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**Abstract** Considerable morbidity and mortality results from the affliction of an estimated 200 million people worldwide by several species of schistosomes; 779 million are exposed to the disease in 74 different countries. Even though anti-parasitic drugs and other control measures, including public hygiene and snail control are available, the advent of an effective vaccine still remains the most potentially powerful means for the control of this disease. The putative vaccine could be administered to small children prior to the time when their contact with infected water is maximal, so as to prevent severe infection in the subsequent years. This review attempts to summarize the status of schistosome vaccine development with special emphasis on functionally important vaccine candidates. The importance of utilizing both murine and nonhuman primate models as a prerequisite for clinical trials is discussed.

## Introduction

Schistosomiasis, also called as bilharzia, is endemic in 74 countries; afflicts an estimated 200 million people (Gryseels et al. 2006) and an additional 779 million individuals are at risk of acquiring this infection (Steinmann et al. 2006). It is now becoming apparent that the impact of schistosomiasis has been underestimated for decades and the real burden of disease is much closer to that of malaria and tuberculosis (Colley et al. 2001; Bergquist et al. 2002). The estimate of 200 million people infected may well be correct but the figures as they relate to morbidity and mortality have to be revised (van der Werf et al. 2003) to include the effects of morbid sequelae such as anemia, growth retardation, and impaired cognitive development as well as rebound morbidity (Bergquist et al. 2005). Disability-adjusted life years for schistosomiasis have been calculated to be 1.7 million (Pink et al. 2005), again these figures need revision (Bergquist et al. 2005; Gryseels et al. 2006).

## Control strategies

Emphasis has been placed on chemotherapy as the preferred method for the treatment of schistosomiasis. However, control programs based on chemotherapy are complicated by the rapidity and frequency of re-infection and the difficulties and expense involved in maintaining these programs over a long term (Bergquist et al. 2005). The possibility that the parasites may develop drug resistance as has recently been reported (Ross et al. 2002; Ribeiro-Dos-Santos et al. 2006), must also be considered. Praziquantel, a drug developed about 30 years ago, is now the only drug available to treat schistosomiasis and, even if the risk of resistance were downplayed, the outcome would be devastating if it did develop (Bergquist et al. 2005). Integrated control programs aimed at limiting schistosomiasis by improving education and sanitation, molluscicide treatment programs to reduce the population of the intermediate snail host and chemotherapy have also had only limited success (Sturrock 2001). Thus, there remains a critical need for the development of alternate approaches to control the disease,

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for example a vaccine (Hagan and Sharaf 2003; Pearce 2003; Da'dara and Harn 2005; Hotez and Ferris 2006).

An efficacious schistosomiasis vaccine would make a significant contribution to current schemes of disease control, particularly if it provides a potent, long-lasting immunity to the disease. Such a vaccine would greatly reduce the need for logistically difficult and expensive drug-based programs which often require political commitment and well-funded public health systems (Gryseels et al. 2006; Hotez and Ferris 2006). Several cost analysis studies have concluded that a vaccine that induces partial resistance to schistosome infection and provides protection for a decade would be less expensive than the current treatment with the drug praziquantel, for children aged 6–15 years (Guyatt and Evans 1995; Evans and Guyatt 1997). Even partial protection against cercarial infection would be a significant advance because a vaccine that reduces worm burden will reduce both the pathology and the transmission rates of the disease. This is because schistosomes, unlike most other infectious organisms, do not replicate within their definitive hosts. Therefore, a sterilizing immunity may not be required for schistosomiasis. In fact, the Scientific Working Group on schistosomiasis at the World Health Organization (WHO) has determined that vaccines that lower adult worm burdens by 50% will be effective in reducing overall morbidity and mortality (Cherfas 1991; Bergquist et al. 2002). In this regard, WHO's Steering Committee on Vaccine Discovery in 1999 recommended a work plan for schistosomiasis whose aim is to produce a vaccine which induces at least 50% resistance to re-infection after drug treatment of a susceptible population. Similarly, a United Nations Development Program/World Bank/WHO's Special Program for Research and Training in Tropical Diseases expert panel (Manila, Philippines, October 2003), again recommended that the most relevant end point assessment for schistosome vaccine efficacy is morbidity reduction; and vaccine effectiveness be determined based on its anti-infection, anti-disease, or anti-fecundity potential.

## Vaccine development—progress and prospects

Development of a schistosomiasis vaccine is made difficult by the immune refractoriness of these parasites, evidenced by their remarkable ability to survive for decades within the bloodstream of immunocompetent hosts. Studies investigating the immune avoidance phenomena (Harnett 2002; 2005) indicate several mechanisms by which schistosomes modify the host's immune responsiveness in their favor. Despite the formidable immune evasion mechanisms employed by schistosomes, it is the consensus view that humans and mice, once infected, generally develop some resistance to further infection (Butterworth 1992). In fact, it is widely believed that the parasite benefits from this partial resistance, or "concomitant immunity", since it serves to limit the pathology to the host and thereby prolonging the productive lifetime of the parasite. The abundant literature on concomitant immunity concludes that natural resistance to re-infection by schistosomes is generally mediated by antibody and antibody-targeting of effector cells, probably macrophages and eosinophils, with T cells also playing an important role in activating the cytotoxic function of these effector cells (Hagan and Wilkins 1993; Brown and Grenfell 2001; Acosta et al. 2002).

It can be argued that a successful vaccine against a complex metazoan parasite like *Schistosoma mansoni* can be developed because (a) the immunization of mice with one dose of irradiated cercariae results in 50–70% protection which can be increased to over 80% with two or three immunizations (Smythies et al. 1996), and (b) human populations following exposure in endemic areas invariably develop some degree of protection naturally (Hagan et al. 1991; Hagan 1992). This view is supported by the fact that veterinary anti-parasite recombinant vaccines against *Echinococcus granulosus* and *Taenia ovis* have been successfully applied in practice (Dalton and Mulcahy 2001; Bergquist et al. 2005; Hein and Harrison 2005).

In this regard, it is important to note the report of the steering committee on vaccines for schistosomiasis of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR). Two laboratories with recognized experience in experimental schistosomiasis independently assessed the protective potential of the six WHO-designated "priority antigens" (paramyosin, glutathione *S*-transferase, fatty acid binding 14 kDa protein, IrV-5, triose phosphate isomerase, and Sm23) and reported that none of them provided the stated goal of 40% protection or better against an experimental challenge infection in mice (TDR newsletter 50, June 1996; Bergquist 1998). Interestingly, the WHO still decided that the further refinement of some of these antigens ought to be done and advance towards human trials (Abath 2000). One of these antigens, *S. haematobium* glutathione *S*-transferase (Sh28GST; Bilhvax), has already been advanced to phase II clinical trials (Capron et al. 2005), even though none of the phase I trials results have been published to date. Taken together, it would be prudent to start the careful examination and assessment of those vaccine candidates that are identified by novel means and examine whether they play important role(s) in the survival of the parasite and are practical in endemic settings.

## Vaccine candidates

During the last two decades, many laboratories have attempted to identify schistosomal antigens that induce at

least a partially protective immune response. More than 100 such antigens have been identified, about 15% of which confer protection of varying degrees and are considered promising though they do not quite reach the level of immunity elicited following vaccination with irradiated cercariae (Bergquist et al. 2002; Hewitson et al. 2005). In some cases, cDNA clones encoding protective epitopes have been characterized, and the identities of several partially protective antigens are known, including paramyosin (Gobert and McManus 2005), glutathione *S*-transferase (Capron et al. 2005), triose phosphate isomerase (TPI; Harn et al. 1992), 14 kDa fatty acid binding protein (Fonseca et al. 2006), Sm23 (Da'dara et al. 2001; Da'dara et al. 2002), GAPDH (Argiro et al. 2000), and Sm-p80 (Siddiqui et al. 2003a; Siddiqui et al. 2003b; Siddiqui et al. 2005a; Siddiqui et al. 2005b). Vaccinations with synthetic or recombinant schistosomal antigens representing selected epitopes have induced partial protection and/or reduced female fecundity in animal models (Balloul et al. 1987; Osburn and Stott 1989; Boulanger et al. 1991a; Soisson et al. 1992; Lebens et al. 2003; Tallima et al. 2003; Veprek et al. 2004). The highest levels of immunity, about 75% protection against cercarial challenge, have been achieved with a truncated portion of a 200 kDa myosin-like protein (rIrV-5) present on the surface of schistosomula (Soisson et al. 1992) and with multiple antigenic peptides (MAPs) containing epitopes from either TPI or Sm23 (Harn et al. 1995). However, TPI may only be suitable for veterinary use due to sequence similarity to human TPI, with a potential for the induction of autoimmune responses; a MAPs vaccine was apparently developed to get around this problem but has been discontinued due to inconsistent results (UNDP/World Bank/WHO-TDR expert panel report; Manila, Philippines, October 2003). The rIrV-5 antigen has not been pursued to the fullest since 1992, unfortunately due to the untimely death of Dr. Mette Strand. A 50–76% protection was also shown with a 74 kDa antigen (Attallah et al. 1999). On the other hand, none of the constructs from some of the aforementioned antigens, when assembled into a variety of single covalent structures incorporating multiple-defined epitopes (capable of inducing T and B cell responses) of *S. mansoni* protected animals from a subsequent challenge infection, indicating that the immune responses elicited were inadequate or inappropriate for parasite killing in vivo (Yang et al. 2000). Furthermore, using an integral membrane protein (Sm23) and several different DNA immunization regimens (e.g., microseeding, gene gun delivery, and intramuscular injections) and co-administration of plasmid DNA encoding both the murine IL-4 and IL-12 genes, protection ranging from 18% to 44% has been recorded (Da'dara et al. 2001; Da'dara et al. 2002). Furthermore, a combination of three plasmids encoding two tegumental antigens (200-kDa glycosylphosphatidylinositol-anchored surface protein commonly referred to as ECL and Sm14) and a muscular antigen (IrV-5) yielded protection levels ranging from 41% to 65% (Nascimento et al. 2002). Using Sm23 in a DNA-prime/protein-boost vaccination regimen, an enhanced Th2 immune response was achieved but the protection levels were greatly reduced from the 36–44% routinely achieved with Sm23 DNA priming alone. These authors concluded that vaccination using Sm23 is associated with a Th1 immune response, and efficacy is diminished using protocols that lessen this Th1 bias (Da'dara et al. 2001; Da'dara et al. 2002; Da'dara et al. 2003). Priming with cDNA encoding Cu/Zn cytosolic superoxide dismutase-pcDNA (SmCT-SOD) and boosting with the same antigen expressed in vaccinia virus resulted in varying degrees of protection (20–85%) in mice (Shalaby et al. 2003; LoVerde et al. 2004). Recent studies utilizing a DNA vaccination strategy with SmCT-SOD induced 39% protection, filamin conferred 50% protection, and glutathione peroxidase (SmGPX) induced no protection compared to controls following challenge with adult worms by surgical transfer (Cook et al. 2004). SmCT-SOD-immunized mice presented with a Th1 response, and filamin-immunized mice showed a mixed Th1–Th2 response (Cook et al. 2004). Recently, oral *Salmonella*-based 14-kDa vaccine conferred 34–49% protection against experimental schistosomiasis (Pacheco et al. 2005). However, individual female worm fecundity did not seem to be affected by this immunization protocol (Pacheco et al. 2005). In summary, there are several antigens which have shown some degree of prophylactic potential, mostly in the murine model. Most of these vaccine candidates were discovered in the last two decades. Recently, there is some renewed emphasis on the discovery of a wide array of antigens via transcriptome analyses (Verjovski-Almeida et al. 2003). Thus, there is great potential in this approach and some of the antigens identified via these newer technologies may help identify additional potential vaccine candidates in the near future.

## In search of functionally important vaccine candidates

Schistosomes interact closely with their host, performing functions such as immune evasion, nutrient uptake, and attachment. We believe that host-exposed, schistosome proteins that undertake such essential functions will be effective targets for a schistosomiasis vaccine. We have targeted a host interactive protein, calpain (=Sm-p80) which plays an important role in the surface membrane renewal of schistosomes (Siddiqui et al. 1993), a phenomenon which is widely considered to be a mechanism employed by hemohelminths to evade host immunity (Young and Podesta 1986). The protein has been shown to be exposed at the host parasite interface (Siddiqui et al. 1993; Braschi and Wilson 2006) and to be naturally immunogenic (Hota-

Mitchel et al. 1999). While the natural immunogenicity of the molecule does not provide protection under conditions of natural infection, it has been demonstrated that it is possible to present calpain to the immune system in such a way as to induce potent immunity in experimental animals (Siddiqui et al. 2003a; Siddiqui et al. 2003b; Siddiqui et al. 2005a; Siddiqui et al. 2005b). Another functionally relevant vaccine candidate is a sugar-transporting protein designated SGTP4 (Skelly and Shoemaker 1996; Jiang et al. 1996). SGTP4 is a host-exposed integral membrane protein that functions to import sugar from the host bloodstream into the body of the parasite (Skelly and Shoemaker, 1996; Jiang et al. 1996). However, not much work has been done on this important vaccine candidate since its discovery in the late 1990s. Recently, tetraspanins (TSP-1/2) present on the apical syncytial surface of *S. mansoni* have been identified and used in a defined vaccine formulation which upon administration provided protection ranging from 29% to 61% and reduction in egg burden from 50% to 61% in mice (Tran et al. 2006). It is speculated that like their human counterparts TSP-1/2 are involved in cell–cell interactions and maintenance of membrane integrity (Tran et al. 2006), and may thus be functionally important antigens as vaccine candidates.

Various immunization regimens (DNA and protein) with Sm-p80 have been tested. Thus far, four "research groups" have independently shown reproducible and consistent protection levels in mice: Nagoya City University Medical School, Nagoya, Japan (Zhang et al. 2001; Ohta et al. 2004); The John P. Robarts Research Institute and University of Western Ontario, London, Canada (Hota-Mitchell et al. 1997; Hota-Mitchell et al. 1999); Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA (Jankovic et al. 1996); and Texas Tech University Health Sciences Center, Amarillo, TX, USA (Siddiqui et al. 2003a; Siddiqui et al. 2003b; Siddiqui et al. 2005a; Siddiqui et al. 2005b). Based on these promising results from these four different laboratories, the UNDP/World Bank/WHO-TDR special panel (Manila, Philippines, October 2003), designated Sm-p80 as one of the priority antigens "with established credentials, needing further development" and Sm-p80 is now considered as one of the "first-tier candidates" by international experts in the field (Bergquist et al. 2005).

Using Sm-p80, a standard immunization regimen has been achieved utilizing Th1 response promoting cytokines as adjuvants in a DNA vaccine formulation in mice. The most effective DNA vaccine regimen (Sm-p80 + IL-2) produced 57% protection in mice (Siddiqui et al. 2003b). The protective immune response in mice appears to be a Th1 type as shown by the generation of IgG2a and IgG2b antibodies (Siddiqui et al. 2003b) and by the synthesis of prototype Th1 cytokines by the antigen-specific proliferating splenic lymphocytes (Siddiqui et al. 2005a). Using a similar immunization strategy, a Th1 type Sm-p80-specific response was generated in baboons and these antibodies were able to kill schistosomula in vitro in the presence of complement (Siddiqui et al. 2005b). Recently, the therapeutic potential of Sm-p80 was also evaluated. Mice were infected with 50 cercariae of *S. mansoni*. Six weeks post-infection, animals were immunized and boosted with a DNA vaccine comprising of Sm-p80 cDNA and IL-2, resulting in a 53% reduction in adult worm burden (unpublished results). Egg production by female worms was decreased by 29%, in "chronically infected"/immunized mice, suggesting that Sm-p80 also has anti-fecundity effects. In summary, our data (Siddiqui et al. 1993; Siddiqui et al. 2003a; Siddiqui et al. 2003b; Siddiqui et al. 2005a; Siddiqui et al. 2005b) and that of others (Jankovic et al. 1996; Ohta et al. 2004) indicate that calpain (Sm-p80) has a great potential as an important vaccine antigen for the reduction of the morbidity associated with both *S. mansoni* and *S. japonicum* infections.

## Should the vaccine efficacy studies performed in mice be replicated in nonhuman primates before clinical trials?

As per WHO-TDR recommendations (>40% protection in mice), antigens like Sm-p80, Cu/Zn cytosolic superoxide dismutase, filamin, and tetraspanins (TSP-1/2) among others are now ready to be taken into human phase I trials. However, it would be prudent if these antigens are not rushed pre-maturely into clinical trials without first testing their prophylactic and therapeutic potential in nonhuman primate systems. There are several reasons for this cautious approach. For example, Lebens et al. (2004) recently pointed out that some of the proposed vaccination strategies based on murine studies could have undesired effects in some individuals if taken to human clinical trials. This is partly because of a developing paradigm suggesting that mechanisms of protection in the permissive mouse model of schistosomiasis cannot completely be generalized to human protection. This opinion is based on the failure to achieve consensus on whether a successful schistosome vaccine should induce Th1 and Th2 responses which contributes to protection/resistance. Furthermore, the recent problems with the clinical trials using anti-CD28 (TGN1412) which had clear efficacy in mice (Vitetta and Ghetie 2006) reinforce the notion that exhaustive and objective testing in nonhuman primates is necessary before embarking onto human clinical trials for a disease as debilitating as schistosomiasis.

Phylogenetically, the great apes are most closely related to humans (*Homo* species). The great apes include chimpanzees (*Pan troglodytes*), orangutans (*Pongo pygmaeus*), gorillas

(*Gorilla gorilla*), and gibbons (*Hylobates lars*). The great apes diverged from the human developmental line over 5 million years ago, with gorillas and chimpanzees being the most recently diverged from humans. These animal models that best reflect the human situation are problematic for use as experimental animal models. This is based on scarcity as some are on the endangered species list and the other reflects cost of usage issues. Yet, a number of these hominoid nonhuman primates have been reported to develop cancers similar to those reported in humans. These include but are not limited to liver, lung, brain, and a variety of hematologic malignancies. Next in the evolutionary development line are the Old World monkeys, which diverged from humans between 15 and 20 million years ago. The Old World monkeys include the drills and mandrills (*Mandrillus*), the common or savannah baboons (*Papio*), gelada baboons (*Theropithecus*), mangabeys (*Cercocebus*), African green monkeys (*Ceropithecus*), and macaques (*Macacca*). The most distantly related to humans are the New World monkeys that diverged approximately 30 million years ago. The New World monkeys are indigenous to South America, while the natural habitats of Old World monkeys include Africa and Asia. The most common New World monkeys that are used in biomedical research investigations include the cotton-topped marmoset or cotton-top tamarin (*Sanguinus oedipus*), common marmosets (*Callithrix jacchus*), owl or aotus monkey (*Aotus trivergatus*), capuchin monkey (*Cebus*), and squirrel monkeys (*Saimiri sciureus*). Although these non-hominoid nonhuman primate species have been under investigation for use as models of infectious disease pathogens and cancer, a number of these species have been reported to develop a variety of infections and malignancies similar to those observed in humans. Perhaps the best use of these monkeys has been in preclinical evaluation as a means of examining safety and the immunologic response to immunotherapies, particular as it relates to vaccination.

Nonhuman primates, especially baboons (*Papio* spp.), currently represent the most relevant nonhuman primate model of human clinical manifestations of the disease. Additionally, the baboon's similarity to humans in ontogeny, immune response, reproductive physiology, placentation, and maternal–fetal antibody transfer as well as their ability to breed well year-round in captivity makes the baboon an excellent model for vaccine efficacy studies (Kennedy et al. 1997; Locher et al. 2001; Wolf et al. 2006; Murthy et al. 2006). Unlike other nonhuman primate models, such as *Macaca* spp. (rhesus and cynomolgus monkeys) which exhibit only three IgG subclasses (IgG1, IgG2, and IgG4), baboons produce human-like four IgG subclasses (IgG1, IgG2, IgG3, and IgG4; Shearer et al. 1999). Unlike rhesus monkeys in which *S. mansoni* infection is self limiting, in baboons, experimental *S. mansoni* infection yields a remarkably high rate of cercarial penetration, fast schistosomula migration from the skin to the lungs and from the lungs to the liver, and development to adult worms; maturation of infecting larvae often exceeds 90% compared with <50% in mice (Nyindo and Farah 1999). Furthermore, baboons develop a human-like acute schistosomiasis syndrome after exposure to the cercariae of *S. mansoni*, manifested by fever, eosinophilia, leucocytosis, cachexia, anorexia, and diarrhea, and these symptoms are coincident with the start of egg deposition in the tissues by newly matured worms (Damian et al. 1992; Farah et al. 2001; Kariuki and Farah, 2005; Alan et al. 2006). Furthermore, baboon as a "protection" model has been utilized successfully by several laboratories to test the efficacy of different schistosome vaccines (Sturrock et al. 1985; Boulanger et al. 1991b; Soisson et al. 1993; Reid et al. 1995; Yole et al. 1996; Kanamura et al. 2002; Kariuki et al. 2004; Kariuki and Farah, 2005; Siddiqui et al. 2005b; Kariuki et al. 2006). For example, immunization of baboons with Sm28GST in the presence of alum resulted in protection from a reduction from 0% to 80% (mean=38%) in the number of parasites and a 33% decrease in female fecundity (Boulanger et al. 1991b). Similarly, inoculation of baboons with IrV-5 elicited a protective response which ranged from 0% to 54% (mean=28%) and associated with a noticeable reduction in the sizes of granulomas (Soisson et al. 1993). Recently, it has been reported that in baboons, efficacy of radiation-attenuated vaccine is not compromised when either prior infection is terminated by drug treatment or the vaccination regime is superimposed on a chronic infection (Kariuki et al. 2006). This is an important finding because in endemic areas, in many cases, a schistosome vaccine may be administered to individuals which have been previously treated with praziquantel. In summary, the baboon can serve as an immensely useful bridge between mouse and human studies (Mountford 2005).

However, one important consideration associated with the use of nonhuman primate studies is the high cost associated with this model; there are thus inherent limitations and less flexibility as it relates to the experimental design due to the inclusion of a small number of animals per variable to be tested. The major concern as it relates to statistical calculations is that the expensive nature of the nonhuman primate model does not allow for traditional statistical power analyses without becoming cost prohibitive, particularly as it relates to vaccine efficacy studies. For the determination of vaccine efficacy, four to six nonhuman primates per group have been an accepted and a widely used number in the field (Watts and Kennedy 1999). The group size of six baboons represents a minimum accounting for at least one atypical responder per group in this outbred population. This fact is illustrated in studies conducted in nonhuman primates for schistosomiasis ($n$=6—Damian et al. 1984; Soisson et al. 1993), influenza virus ($n$=4—Bot et al. 1999), dengue-2 virus ($n$=3—Putnak et al. 2003), HIV/

SIV ($n$=5—Wee et al. 2002; $n$=6—Amara et al. 2001; Horton et al. 2002), Ebola virus ($n$=4—Sullivan et al. 2003) and many others. Statistically significant data have been obtained using four to six animals in many vaccine efficacy studies, especially when the outcomes are response or no response, or protection or no protection.

## Conclusions and future directions

Undoubtedly, development of an efficacious schistosomiasis vaccine would be a medical breakthrough. It is becoming clear that the most germane end point measurement for schistosomiasis vaccine efficacy is morbidity reduction. In this regard, emphasis should be placed on testing the therapeutic potential of antigens in addition to the conventional prophylactic efficacy estimation studies. Most of schistosome vaccine candidates confer 30–50% protection in the mouse model system. Thus, there is a great need to identify new antigens which may be able to induce higher and more consistent and durable levels of protection than the usual <50%. More research is required on the development of novel adjuvant vehicles as well as cocktail vaccine formulations to enhance protection levels to at least induce protection that ranges from 70% to 80%, as has been recorded with radiation-attenuated vaccines. Furthermore, when designing immunization regimens for clinical trials, data obtained through studies in the murine model should be used with caution and some thought should be given to other variables (e.g., human correlates, Th1–Th2 balance, etc.) to achieve optimal results in the human system. It is our opinion that before embarking onto clinical trials, every credible schistosome vaccine candidate should first be tested in a nonhuman primate model system. However, nonhuman primates should be used sparingly and only when justified scientifically (Dowsing and Kendall 2007). Finally, there is an urgent need for substantial infusion of resources from both public and private sectors for research and development of a schistosomiasis vaccine.

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