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Review

A vaccine against Asian schistosomiasis

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Abstract

There is continued transmission of schistosomiasis japonica in China and Philippines despite highly effective control programs that focus on the application of the highly effective drug praziquantel (PZQ). The massive Three Gorges Dam across the Yangtze River in Southern China, soon to be completed, is expected to significantly increase schistosomiasis transmission and introduce the disease into areas currently unaffected. After long-term experience it is generally accepted that PZQ chemotherapy, although the cornerstone of current control programs, does have significant limitations. Furthermore, efficient drug delivery requires a substantial infrastructure to regularly cover all parts of an endemic area. Although there is not yet clear-cut evidence for the existence of PZQ-resistant schistosome strains, decreased susceptibility to the drug has been observed in several countries. As a result, a protective vaccine represents an essential component for the long-term control of schistosomiasis. This article briefly reviews aspects of anti-schistosome protective immunity that are important in the context of vaccine development. The current status in the development of vaccines against *Schistosoma japonicum* will then be discussed as will new approaches that may improve on the efficacy of available vaccines, and aid in the identification of new targets for immune attack. With new and extensive data becoming available from the *S. japonicum* genome project, the prospects for developing an effective vaccine are encouraging. The challenges that remain are many but it is crucial that the momentum towards developing effective anti-schistosome vaccines is maintained.

Keywords: *Schistosoma japonicum*; Vaccine; Transmission blocking vaccine; Paramyosin

1. Introduction

New data [1] indicate that, in terms of morbidity and mortality, schistosomiasis, represents an even greater disease burden than previously appreciated. Over 250 million people and significant numbers of domestic livestock animals are infected and 280 000 individuals die as a direct result of schistosomiasis infection in sub-Saharan Africa alone annually [1]. Despite the availability of a highly efficient drug, praziquantel (PZQ), there is considerable spreading of schistosomiasis into new endemic areas [2]. The construction of dams and the development of important irrigation schemes are often followed by epidemic outbreaks of the disease. In regards to the Asian schistosome, *Schistosoma japonicum*, the massive Three Gorges Dam

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across the Yangtze River in Southern China, soon to be completed, is expected to significantly increase schistosomiasis japonica transmission and introduce the infection into areas currently unaffected [3]. After some 20 years experience it is generally agreed that chemotherapy, although the mainstay of current control programs, does have significant limitations. In particular, mass treatment does not prevent re-infection. This rapidly occurs in exposed populations in most endemic areas so that within a period of 6–8 months following chemotherapy the prevalence returns to its baseline level. Efficient drug delivery requires a substantial infrastructure to regularly cover all parts of an endemic area. This makes chemotherapy an expensive and often impractical approach [4]. Furthermore, despite the fact there is not yet clear-cut evidence for the existence of PZQ-resistant schistosome strains, decreased susceptibility to the drug has been observed in several countries, although not as yet in China or Philippines [5–7]. Vaccines (for use in livestock animals, particularly bovines and humans) in combination with other control strategies, including the use of new drugs, are needed to make elimination of the disease possible [8].

Vaccination can be either targeted towards the prevention of infection or to the reduction of parasite fecundity. A reduction in worm numbers is the gold standard for anti-schistosome vaccine development but, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability appears also entirely relevant. Indeed the only schistosome vaccine, Bilhvax, that has entered human (phase II) clinical trials, induces immune responses with anti-fecundity effects against female *S. haematobium* [9]. This review considers very briefly some aspects of anti-schistosome protective immunity that are important in the context of vaccine development. Current progress towards the development of a vaccine against *S. japonicum* is highlighted, as are new approaches that may improve on the efficacy of available vaccines and aid in the identification of new targets for immune attack. Comprehensive reviews of the area are available [8–12].

2. Mechanisms of immunity

Research developed on schistosomes for over 20 years has led to the identification of in vitro mechanisms of protective immunity against infection or re-infection in animal models and in humans. The most significant contributions of these studies have been the demonstration in vivo of the protective role of IgE [13] and eosinophils [14]. In humans, epidemiological correlations supporting these experimental observations and arising from several different studies in various parts of the world strongly suggests that IgE may be one of the key components of protective immunity [15,16].

Together with IgE, high levels of IgG4 are produced during schistosome infections. Preliminary evidence was first reported of a significant correlation between susceptibility to re-infection of *S. haematobium* in humans and increased production of IgG4 to defined schistosome antigens [17]. In fact, in subsequent studies, elevated production of IgG4 and IgG2 antibodies was consistently associated with increased susceptibility to re-infection [18]. From these studies, it was concluded that immunity to re-infection was more closely related to the IgE/IgG4 balance than to the absolute level of each isotype. Similar findings have been obtained with *S. haematobium* and *S. japonicum* infections [12,16].

The clinical expression of immunity to schistosome infection is obviously not simply determined by the mere balance between IgE and IgG4 antibodies. It cannot exclude the participation of additional mechanisms observed in experimental models [19]. The evidence for additional protective mechanisms operating in suggests a potential protective role for IgA antibodies in human schistosomiasis [20] supported by a series of convergent correlation studies in several parts of the word including *S. haematobium* and *S. japonicum* infection. The effector function of IgA antibodies appears to be associated with a decrease in female worm fecundity and egg viability [21].

Although the existence of highly complex networks of regulation does not allow any attempt at oversimplification, it is by and large, still the case that clinical expression of protective immunity to

schistosomes in human is largely associated with a Th2 profile of immune response. Within this, distinct mechanisms (either IgE or IgA dependent) might be implicated in the control of infection or of egg induced pathology [9,22]. However, recent field work from Philippines [23] supports the contention that resistance to reinfection is associated with a predominant Th1 response to soluble worm antigen preparation (SWAP) whereas IL-10 production in response to SWAP was correlated with susceptibility. Human immunity against schistosomiasis exists. Clearly the elucidation of the human protective immune mechanism is a research imperative.

3. Vaccines against *S. japonicum*—basic considerations

Schistosomiasis japonica is endemic in Southern China and Philippines [24]. The control of schistosomiasis requires an integrated approach involving large-scale population-based chemotherapy in addition to environmental and behavioral modification [25]. Further, population movements [1] and the construction of dams already referred to, have contributed to the spread of schistosomiasis into new areas. There is an important role for a vaccine to provide long-term prevention against Asian schistosomiasis. Unlike *S. mansoni* or *S. haematobium*, mammals other than humans (such as dogs, bovines and pigs) can act as definitive hosts for *S. japonicum*. Zoonotic transmission adds to the complexity of control programs, but may provide opportunities for novel approaches in vaccine development to prevent human disease. When available for wide-scale use, it is envisaged that the vaccine would be applied in the first instance, at least in China, in water buffalo reservoir hosts (a 'blocking' vaccine to impact on human transmission) and then, perhaps, if required, clinically (to prevent or reduce disease).

Recent progress has provided the basic framework for the development of an effective vaccine against this complex parasite. Schistosomulum lifecycle stage antigens are likely to be major vaccine candidate targets of protective immune responses. Vaccination with radiation-attenuated cercariae induces significant levels of resistance to *S. japonicum* challenge in mice, rats, rabbits, sheep and bovines [12]. Worm burden reduction ranges from 40 to $>85\%$—with less protection evident in mice than rabbits or larger animals. The initial protection is conferred by Th1 cellular immunity with lymphoid proliferation in regional and mediastinal lymph nodes. Subsequent vaccination (at least in mice) did not appear to confer additional protection. Appropriately-timed passive transfer of sera from vaccinated mice can also confer protection. This protection is antibody-mediated, with predominantly IgG (less so by IgM) antibodies acting on the lung schistosomula stage. IgE depletion did not influence protection in these studies. Some later studies suggest that both Th1 and Th2 responses may contribute to protection [12].

Human immunity to *S. japonicum* has predominantly been assessed by re-infection and immune-correlative studies. Acquired immunity to *S. japonicum* develops with age [12,16]. A high IgG4/IgE ratio to parasite antigens correlates susceptibility to re-infection, whereas IgE excess correlates with resistance to re-infection [26]. Further, peripheral blood mononuclear cells taken from resistant individuals in China produce significantly greater amounts of IL-10 in response to parasite extracts and recombinant antigens in vitro [27]. Preliminary field studies from Philippines suggest that IgE antibodies to a 22.6 kDa tegument-associated antigen (Sj22.6) are associated with resistance to reinfection [12]. It should be stressed that whereas much of our understanding of the effector mechanisms and clinical expression of immunity against *S. japonicum* has been extrapolated from the extensive studies on *S. mansoni* and *S. haematobium*. This may prove not to be the case, given the extensive biological and molecular differences that exist between the causal parasite species [28].

4. Target antigens

While offering in some cases high levels of protection, the irradiated cercarial vaccine is impractical, cumbersome and impossible to standardize. Additionally, the protection conferred is

Table 1
Major *Schistosoma japonicum* vaccine candidates

Antigen (native or recombinant protein)	Short form	Size (kDa)	Stage expressed	Biological function	*Claimed protection (%)	
					Mouse	(Other)
Paramyosin (native)	Sj97	97	Somula Adult	Contractile protein + others	27–86	31–48 (sheep/cattle)
Paramyosin (recombinant)	recSj97	97	Somula Adult	Contractile protein + others	20–60	17–60 (buffalo/pigs/sheep)
Triose phosphate Isomerase (native)	TPI	28	All stages	Enzyme	21–24	
Integral membrane protein (recombinant)	Sj23	23	Adult	Membrane protein		32–59 (buffalo/cattle/sheep)
Aspartic protease (recombinant)	SjASP	46	All stages	Digestion of haemoglobin	21–40	
Calpain large subunit (recombinant)	r-calpain	80	Adult	Protease	40	
Glutathione *S*-transferase (recombinant)	Sj28-GST	28	All stages	Enzyme	0–35	33–69 (buffalo/sheep)
Glutathione *S*-transferase (recombinant)	Sj26-GST	26	All stages	Enzyme	24–30	25–62 (buffalo/pigs/sheep)

*Reduction in worm burdens. Significant egg reduction also recorded with the majority of candidates (see Refs. [12,31,36].

species and often strain-specific. Further, vaccination, at least of mice, with gamma-irradiated *S. japonicum* cercariae suggests that this model may not be as effective as vaccination with UV-attenuated cercariae [29]. Considerable efforts have been aimed at the identification of relevant schistosome antigens that may be involved in inducing protective immune responses, with a view to developing either a recombinant protein, synthetic peptide or DNA vaccine [12]. Coordinated laboratory and field research have identified a set of well-defined *S. japonicum* molecules with protective potential (Table 1).

One leading candidate is paramyosin, a 97 kDa myofibrillar protein with a coiled-coil structure that is found exclusively in invertebrates. It is expressed on the surface tegument of lung-stage schistosomula and may function as a receptor for Fc [30]. Native and recombinant paramyosin confer significant protection (approx. 35% decreased worm burden and 45% decreased liver egg burden) against *S. japonicum* in mice and buffaloes [31]. There is greater than 95% homology between the paramyosin genes of *S. japonicum* (Chinese and Philippine strains), *S. haematobium* and *S. mansoni* [31]. This may facilitate development of a 'consensus' molecule as a vaccine against all three human pathogens, should efficacy be improved. Currently, mathematical modelling of the likely benefits of rec-Sj-97 at its current level of efficacy as an anti-fecundity vaccine suggests, it would prove a useful adjunct to existing control programs [1,32].

A calcium-activated neutral proteinase (calpain) may be another 'consensus molecule' vaccine. It has been identified in *S. mansoni* and *S. japonicum* and has been localized to the extracellular domain [33]. Immunization of BALB/c mice with purified recombinant *S. japonicum* calpain (r-calpain) [34] emulsified in complete Freund's adjuvant resulted in a significant reduction in the number of recovered worms and also in egg production per female worm was observed [34]. These results point to *S. japonicum* calpain as a vaccine candidate for both

worm killing and disease prevention, possibly through the induction of a strong Th1-dominant environment in immunized mice. Vaccination of water buffaloes or some other natural host for *S. japonicum* with r-calpain is clearly the next step in determining its true vaccine potential. The protein is recognized by sera from infected patients so it may also prove useful as a target for immunodiagnosis of human schistosomiasis [33].

The *S. mansoni* and *S. japonicum* 22.6 kDa (Sm22.6 and Sj22.6) tegument-associated antigens may also provide useful vaccine targets. Whilst the function of this family of proteins remains unknown, they are expressed near the surface of lung-stage schistosomula [35] and specific IgE and IgA antibodies appear associated with resistance to reinfection in human studies [12]. Mouse studies of bacterially expressed and purified recombinant Sj22.6 have demonstrated specific IgG and IgE production but no protection [35]. Also, vaccination with *Escherichia coli* and baculovirus-expressed recombinant *S. japonicum* aspartic protease-cathepsin D—generated high levels of specific antibodies but only a limited level of protection [36].

More encouraging results have been obtained with recombinant 26-kDa GST of *S. japonicum* which induces a pronounced anti-fecundity effect, as well as a moderate but significant level of protection in terms of reduced worm burden. The molecule is capable of stimulating anti-fecundity immunity in mice (up to 59% decrease in liver eggs) [37] and pigs (54% decrease in liver eggs) [38] following challenge infection with *S. japonicum*. Similar vaccination experiments have been carried out on water buffaloes (*Bos buffelus*), the major reservoir for transmission of schistosomiasis japonica in China, using purified reSjc26GST in order to investigate its vaccine potential [39]. Anti-Sjc26GST antibodies were produced in the immunised buffaloes and, following challenge with *S. japonicum* cercariae, a small but significant reduction in worm numbers was evident in vaccinated when compared with control animals. The typical anti-fecundity effect was manifest, characterised by a significant decrease in faecal egg output and eggs deposited in host tissues with those in the liver and intestine being reduced by approximately 50%. In addition to the anti-fecundity effect, re-Sjc26GST reduced by nearly 40% the egg-hatching capacity of *S. japonicum* eggs into viable miracidia.

Schistosomes utilise haemoglobin from ingested host erythrocytes as their main source of amino acids and the enzyme cathepsin D aspartic protease plays a key role in maturing and adult schistosomes in the proteolysis of host haemoglobin from ingested erythrocytes [40]. Mice were vaccinated with recombinant *S. japonicum*, expressed in both insect cells and bacteria, in order to evaluate the vaccine efficacy of the schistosome protease [36]. Mean total worm burdens were significantly reduced in vaccinated mice and significant reductions in female worm burdens were also recorded but vaccination did not reduce fecundity. Vaccinated mice developed high levels of antibodies (predominantly IgG1, IgG2a and IgG2b isotypes), but there was no correlation between antibody levels and protective efficacy. Immune sera from vaccinated mice did not inhibit the in vitro degradation of human haemoglobin by the recombinant protease, and passive transfer of serum or antibodies from vaccinated animals, before and after parasite challenge, did not significantly reduce worm or egg burdens in recipient animals. These results suggested that antibodies may not play a key role in the protective effect elicited, and that protection may be due to a combination of humoral and cell-mediated responses. A subsequent study showed that vaccination of mice with the schistosome protease induced a mixed Th1/Th2 cytokine response [41].

Side-by-side comparisons of candidate defined-antigen vaccines have been carried out in sheep, another natural host for *S. japonicum* [42]. Recombinant antigens selected for testing were: the isoforms of glutathione-*S*-transferase (Sj28GST and Sj26GST), the large hydrophilic domain of Sj23, the homologue of the protective *S. mansoni* membrane antigen Sm23 and a 3′ fragment of *S. japonicum* paramyosin. In addition, Chinese strain *S. japonicum* native paramyosin and GST were purified and used for vaccination. Antigens were co-administered with Freund's adjuvant or BCG. Also, examined was the effect of co-administration of native unfractionated GSTs with keyhole limpet

haemocyanin (KLH). KLH shares a cross-reactive protective epitope with schistosomes. Partial but significant protection was obtained with each of the antigens tested. Less protection was obtained with the recombinant fragment of *S. japonicum* paramyosin compared with the native protein. Co-administration of native GST and KLH was no more effective than vaccination with either antigen alone. Although encouraging levels of protection against *S. japonicum* were demonstrated using each of these antigens, further work is needed to optimize vaccine delivery and vaccination schedules.

To summarize, all of the proteins mentioned above have shown some promise as candidate vaccines although they generally produce relatively modest reductions in worm and egg burdens. In the longer term, they may not prove to be the most effective vaccine antigens and it is important, therefore, to identify new target antigens and to explore alternative vaccination strategies to improve vaccine efficacy.

5. DNA vaccines

DNA vaccination offers many potential advantages including cost-effective production, thermal stability and the ability to induce a wide variety of immune responses including induction of cytotoxic T-lymphocytes [43,44]. DNA vaccination has been explored in a variety of parasite, including helminthic, infections [44,45] with significant reductions in parasite burdens being demonstrated in animal models of some of these infections [46–51]. Although a number of reports on schistosomes have reported success in using DNA constructs to induce encoded antigen-specific responses [52–55], there has been limited success in inducing protective immunity against *S. japonicum*. One group [56] has achieved some encouraging results using a DNA cocktail in mice. The cocktail vaccine comprising four DNA plasmids encoding four different *S. japonicum* antigens, Sj62, Sj28, Sj23 and Sj14-3-3 induced significant resistance against *S. japonicum* cercarial challenge infection in two of three experiments. The same group [57] obtained some protection in the field as well as in the laboratory in sheep and water buffalo with DNA vaccine formulations of Sj28GST and Sj23 and in field trials with both DNA vaccines in cattle [58]. Protection against challenge infection in mice has also been obtained with a nucleic acid vaccine (Sj31BIN) combined with IL-12 as adjuvant [59] and with a partial cDNA expression library [60].

A prime-boost strategy of DNA vaccination followed by recombinant modified vaccinia virus Ankara (MVA) encoding pre-erythrocytic stage and erythrocytic antigens has induced sterilizing immunity to malaria [61,62]. A DNA prime-protein boost strategy using the *Plasmodium falciparum* 175 kDa erythrocyte binding protein induced a significant reduction in parasitaemia in *Aotus* monkeys [63]. Similar prime-boost strategies hold much promise for other apicomplexan parasites such as *Theileria* spp. [64]. Prime-boost vaccination along similar lines may prove valuable against schistosomiasis.

Unlike vaccination with native or recombinant protein [31,65], DNA vaccination with paramyosin has yielded conflicting results. Early studies demonstrated specific antibody production without protection [54]. However, another group [66] recently demonstrated 35–40% reduction in worm burden and 50–80% reduction in visceral egg burdens following intramuscular DNA vaccination into the *tibialis anterior* muscle of a different inbred mouse strain. The authors concluded that the differences in mouse strain and site of injection could account for the contrasting results. It is well described that intra muscular immunization (IMI) of DNA vaccines into *tibialis anterior* induces significantly greater immune responses than IMI into the quadriceps muscle in mice [43]. DNA prime-protein boosting with paramyosin is an attractive prospect.

DNA vaccination with either the 22 kDa or 23 kDa *S. japonicum* tegument-associated antigens [52,53] has induced high titres of specific murine antibodies without protection. This is despite manipulation of the route of administration and fusion of the antigen cDNA to an Ig κ-chain secretory leader sequence [53]. Equally, DNA vaccination with a 62 kDa fragment of *S. japonicum* myosin did not induce protection in several

strains of mice despite cationic lipid adjuvant and additional CpG motifs [52].

6. Antigen discovery

Vaccine candidate proteins are often secreted by or anchored on the surface of pathogens, and they usually possess N-terminal hydrophobic signal peptides or signal anchors that direct traffic of the protein through the secretory pathway to the cell surface. Proteins that are secreted by or anchored on the surface of the intramammalian stages of schistosomes are exposed to host tissues and thus present as potential candidate molecules for the development of new intervention strategies. Signal peptides are usually 15–30 amino acids and consist of a basic N-terminus, a hydrophobic center and a polar C-terminus. While they share a similar architecture, high levels of degeneracy make them difficult to identify from primary sequence alone and they cannot be cloned by degenerative PCR-based methods. Signal peptides initiate export of the precursor proteins across the endoplasmic reticulum. Signal sequence trap (SST) is a recently described technique that allows selective cloning of cDNAs that encode open reading frames with an N-terminal signal peptide that directs surface expression of a reporter gene product that lacks its endogenous signal peptide. cDNA libraries are constructed in a plasmid vector so that they fuse in-frame with a reporter gene that lacks its endogenous signal sequence.

Given the widespread interest in interactions between host tissues and secreted and surface proteins of pathogens, SST provides an ideal platform by which to selectively screen parasite genomes for mRNAs that encode proteins with signal peptides. An alkaline-phosphatase signal sequence trap method [67] has been used to isolate sequences encoding secreted and transmembrane proteins from adult schistosome worm cDNA. Amongst 18 clones identified and sequenced, two encoded novel tetraspanin-like proteins, five sequences had no known homologues and several others were homologous to known *S. mansoni* ESTs. It remains to be seen whether any of these molecules are expressed in the tegument and might therefore warrant further investigation as potential vaccine candidates.

B-cell antigenic determinants appear to be dependent on the conformational integrity of the molecule in question. Conformational epitopes may be important in generating protective immunity against parasites. One example is the recent determination that the protection conferred against experimental cystic hydatid disease by the EG95 vaccine, a recombinant oncospheral protein, relies on conformational integrity rather than linear epitopes [68]. Indeed, the absence of tertiary structure and therefore conformational epitopes in the recombinant Sj22.6 vaccine is one possible explanation for the apparent lack of protection associated with this molecule [35]. Clearly further evaluation of synthetic protein vaccines may need to take conformational epitopes into consideration. As well, improved efficacy of anti-schistosome vaccines may require more powerful adjuvants than those currently available for clinical use. A Lewis-type carbohydrate-Lacto-*N*-fucopentose III (LNFPIII)-found in extracted *S. mansoni* egg antigens, has been recently demonstrated to be a potent inducer of a Th2-type response [69]. LNFPIII can also act as an adjuvant by inducing antibody against coupled protein antigen (human serum albumin) [69]. This molecule might prove useful for future development as an adjuvant for application with schistosome vaccines.

7. Schistosome vaccines-quo vadit?

Both *S. mansoni* [(http://www.tigr.org/tdb/e2k1/sma1/) (http://verjo18.iq.usp.br/schisto/)] and *S. japonicum* (http://schistosoma.chgc.sh.cn) have become the subject of major genome characterization initiatives, through support from the Tropical Diseases Research Program of the World Health Organization and other agencies. This has resulted in a rapid expansion of knowledge of the schistosome genome including the development of cDNA libraries from different developmental life cycle stages and the generation of significant numbers of expressed sequence tags (ESTs) from *S. mansoni* and *S. japonicum*. It is likely that a number of novel vaccine candidates will be identified for future study as a result of this important

work. In the case of *S. japonicum*, this new research has generated 43 707 new expressed sequence tags derived from adult *S. japonicum* and their eggs; these have been assigned to 13 131 gene clusters [70,71] that probably encode the entire transcriptome of the parasite. Six hundred and twelve sequences have complete open reading frames, the majority of which has sequence homology with known proteins. Wide scale post-genomics research, focusing on these newly discovered genes and their expression products, including the characterisation of new candidate vaccine molecules could now be undertaken. The EST data indicate that, remarkably, *S. japonicum* encodes receptors for mammalian insulin, progesterone, various cytokines and neuropeptides [70], suggesting that host hormones orchestrate schistosome development and maturation, and suggesting also that schistosomes modulate anti-parasite immune responses through inhibitors, molecular mimicry and other strategies. Receptors such as these may prove to be excellent candidates as vaccine targets.

Taking the breadth of consolidated, international efforts to generate anti-schistosome vaccines, there is optimism therefore that these endeavors will prove successful. When developed and employed, anti-schistosome vaccines will not be a panacea. They need to be regarded as one component, albeit a very important one, of integrated schistosomiasis control programs that complement existing strategies including chemotherapy and health education. Major challenges that remain include declining numbers of young scientists attracted to the area of schistosome vaccine development and the decrease in funding for helminth research in general. Major sponsors are crucial to provide financial support for the upscaling production of defined vaccine candidates, their evaluation in human clinical trials or large scale field trials of *S. japonicum* vaccines in water buffaloes. These challenges have been highlighted also by others [72,73] but it is important to reinforce these views. With the new and extensive data becoming available from the *S. japonicum* genome project (and complementary studies on *S. mansoni* [74]), the prospects for developing effective anti-schistosome vaccines are bright, and it is crucial that this momentum is maintained.

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